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Description of a new species of the genus *Pieris* (Lepidoptera Pieridae) from North Yunnan, China

Teruo TADOKORO[1)], Tsutomu SHINKAWA[2)] and WANG Min [3)]

[1)] 7-5-35 Kinuta, Setagaya-ku, Tokyo, 157-0073 Japan
[2)] 2845-5 Iwasaki, Sueyoshi-cho, Soo-City, Kagoshima, 899-8601 Japan
[3)] Laboratory of Insect Ecology, South China Agricultural University, Guangzhou, China

Abstract Based on molecular phylogeny and morphological characteristics, *Pieris shangrilla* sp. nov. is described from North Yunnan. The new species resembles *Sinopieris davidis* (Oberthür 1876) and *Sinopieris venata* (Leech 1891), but is distinguished from them by the wing markings, androconia and the shape of the uncus and valva of the male genitalia.

Key words Systematic, Taxonomy, *Sinopieris*, *Pieris*, *davidis*, *venata, dubernardi*, molecular phylogeny, *mt*DNA, *Pieris shangrilla* sp. nov.

Introduction

During our recent investigation and subsequent phylogenetic analyses of the Pieridae of East Asia, we found a new butterfly, which resembles *Sinopieris davidis* and its related species/subspecies. The butterfly was collected in North Yunnan of China in May. After careful observation of the wing markings, androconia and male genitalia, we recognize some distinct morphological characteristics of the butterfly.

Huang (1995) described the genus *Sinopieris* with a new species *Sinopieris gongaensis* as the type species for "*dubernardi*-group (*dubernardi* Oberthür 1884 and its related subspecies)", and revised its taxonomy, changing the genus from *Pieris*/*Pontia* to *Sinopieris*. *Sinopieris gongaensis* was later treated as a synonym of (*davidis*) *venata* Leech 1891. Thereafter, *dubernardi*, *davidis*, *venata* and related taxa have been included in the genus *Sinopieris*. Although the new butterfly resembles *Sinopieris davidis* very closely, part of its wing markings do not accord with the description of the genus *Sinopieris*. The androconia and male genitalia also indicate that the butterfly is different from *davidis* and *venata*.

Furthermore, phylogenetic analyses by *mt*DNA genes (16SrRNA) indicate that the butterfly had an independent molecular lineage, and no close relationship with *davidis* and/or even *dubernardi*.

Based on the distinct morphological characteristics and the results of phylogenetic analyses, we describe this butterfly as a new species under the genus *Pieris* Schrank 1801.

Materials and Methods

Specimens of *Pieris shangrilla* (Fig. 1),**Sinopieris davidis* ssp. (Fig. 2), *S. venata* (Fig. 3) and *S. dubernardi* (Fig. 4) we examined were obtained from reliable sources. *P. shangrilla*, **S. davidis* ssp. and *S. dubernardi* were collected in Zhongdian district (called as Shangrila County) in Yunnan Province of China (altitude 3,100 m) in May 2009/2012, and *S. venata* was collected at Danba in Sichuan Province of China (altitude 2,380 m and 80 km north of its type locality) in June, 2005. The photographs of type specimens were provided by NHM (BMNH) in London, and Senckenberg Naturhistorische Sammlungen in Dresden. Androconia were removed from the male forewing upper side cell and observed by optical microscope of 400 magnifications. Male genitalia were dissected and observed by stereomicroscope after removing skins and muscles by 10% KOH solution followed by PBS solution.

Mitochondrial DNA genes we analysed were 16S ribosomal RNA sequence 909 bp, extracted from the legs of *shangrilla*, **davidis* ssp. and *dubernardi* within one month from the collecting dates and amplified by primers. Shinkawa analysed them by 3130 sequencer of ABI after Cycle sequence treatment to the amplified region. Molecular relations were made by Neighbour-joining method with the software "Paup 4.0b10" at Faculty of Science, the University of Tokyo. The evolutionary distances were computed by Kimura's two-parameter method, and integrated into the existing NJ Tree of *Pieris* Group (*mt*DNA-16SrRNA gene made by Shinkawa and Nonaka 2010).

The holotype specimen of *Pieris shangrilla* sp. nov. will be deposited in the South China Agricultural University in Guangzhou, and paratype specimens will be deposited in the University Museum of the University of Tokyo, the Research Institute of Evolutionary Biology in Tokyo and NHM in London.

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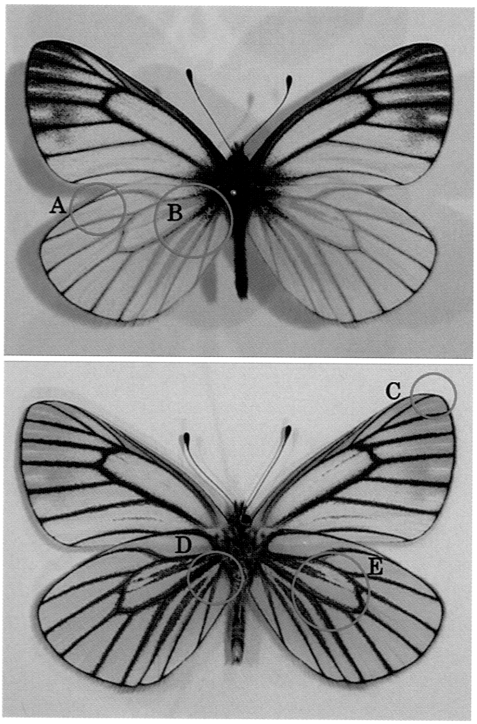

Fig. 1. *Pieris shangrilla* sp. nov. Holotype ♂ from Zhongdian district, Yunnan Prov. (Alt. 3,100 m).

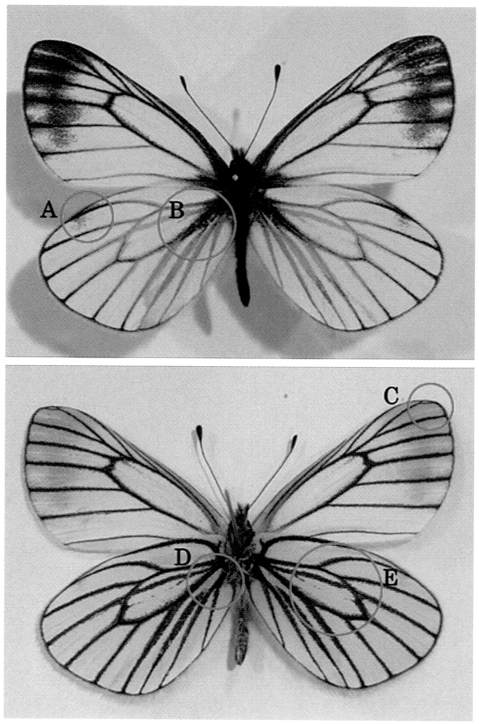

Fig. 2. **Sinopieris davidis* ssp. ♂ from Zhongdian district, Yunnan Prov. (Alt. 3,100 m).

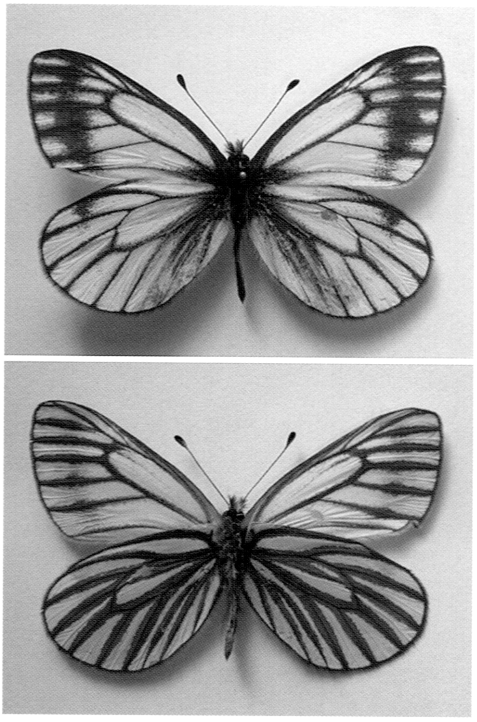

Fig. 3. *Sinopieris venata* ♂ fron Danba, Sichuan Prov. (Alt. 2,380 m).

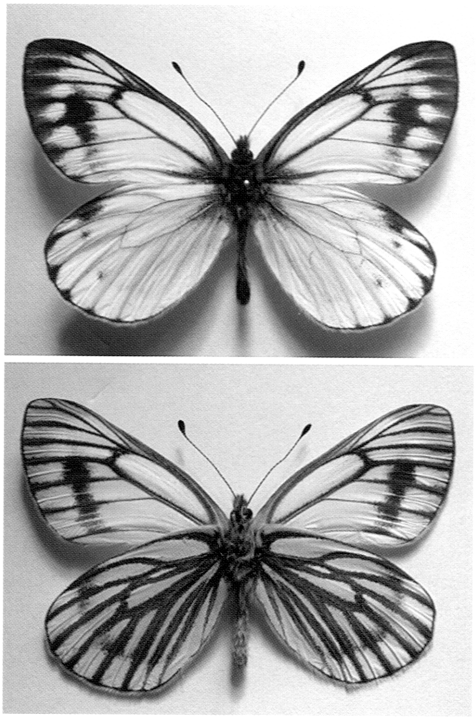

Fig. 4. *Sinopieris dubernardi* ♂ from Zhongdian district, Yunnan Prov. (Alt. 3,100 m).

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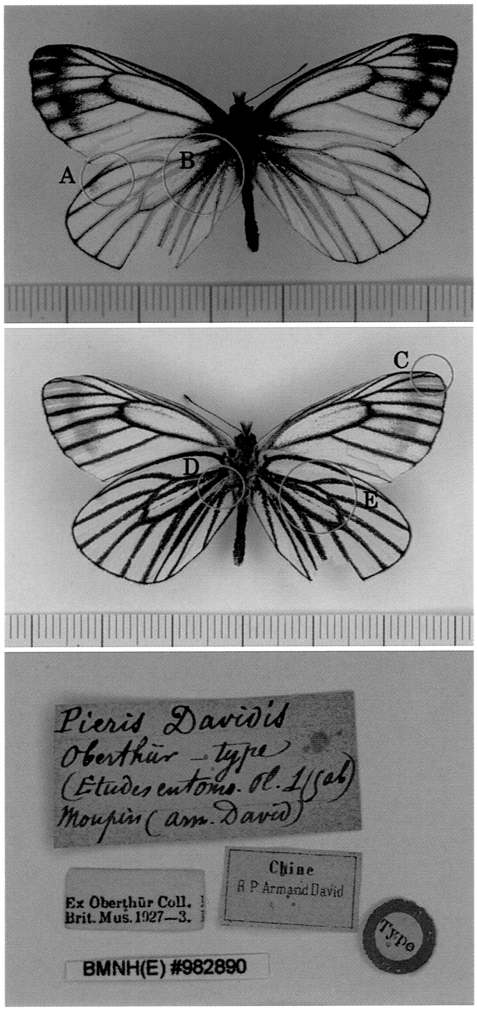

Fig. 5. Type specimen of *Sinopieris davidis* Oberthür, 1876 ♂ (©NHM).

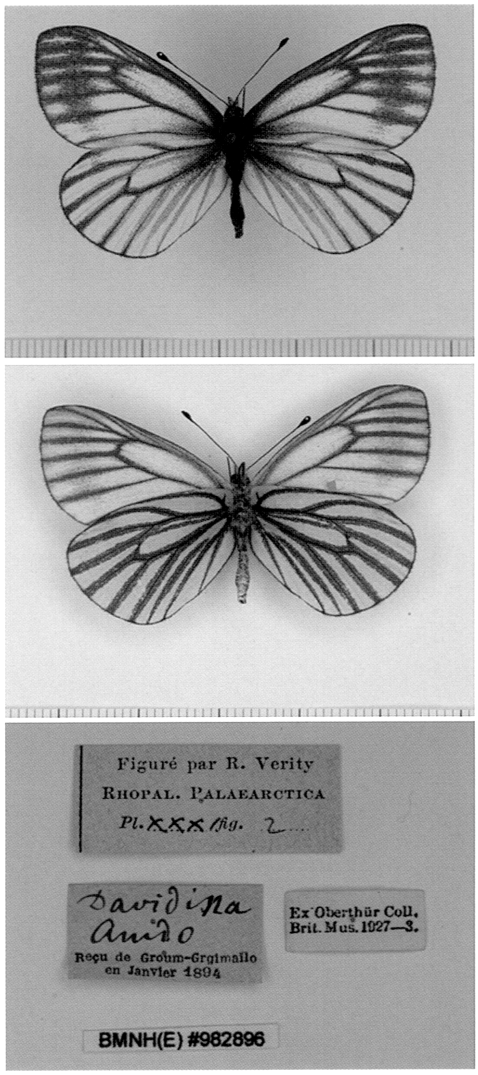

Fig. 6. Type specimen of *S. davidis thibetana* Verity, 1907 ♀ (©NHM).

Description

Pieris shangrilla Tadokoro, Shinkawa & Wang, sp. nov. (Fig. 1)

Holotype ♂. Zhongdian district, Yunnan Prov., China (altitude 3,100 m), 02 V, 2009; Paratypes 7♂. same labels as holotype. 9 ♂. same locality, 20-22 V, 2012.

Etymology. The specific name "*shangrilla*" is derived from the type locality of the new butterfly, "Zhongdian district" known as "Shangrila County".

Wing expanse: 50 mm–56 mm for male.

Wing markings (male): Wings white with the venation blackish. Particularly, median vein and forewing vein 3 to 6 broadly bordered by black scales. Basal area of upperside black. Broad and black submarginal band continues from the apex to space 2 of upper side forewing, but does not continue to hindwing. No apical markings on hindwing spaces 6 and 7. Forewing underside white, with apex pale yellow. Ground color of hindwing underside pale yellow. Only basal part of space 8 vivid yellow. Two black streaks running in the cell of hindwing underside. Lower streak rather thicker than upper one but both streaks become thinner and often disappeare within discal area.

Androconia (Fig. 9). Lamina broad and very broadly bifurcate. Scent bag large.

Male genitalia (Fig. 10). Tegumen posteriorly raised up (A). Uncus thick and long (B). Valva is large. Anterior margin linear (C) and posterior margin projecting (D).

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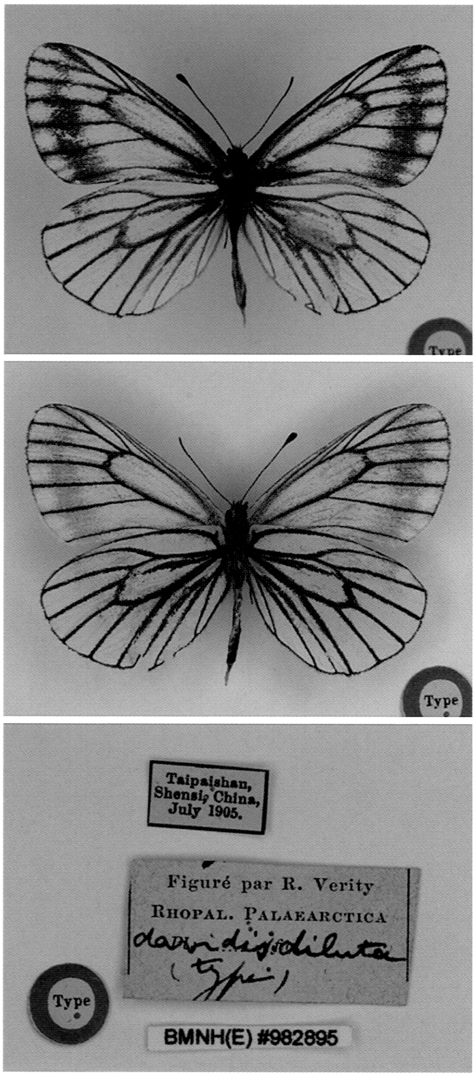

Fig. 7. Type specimen of *S. davidis diluta* Verity, 1911 ♂ (©NHM).

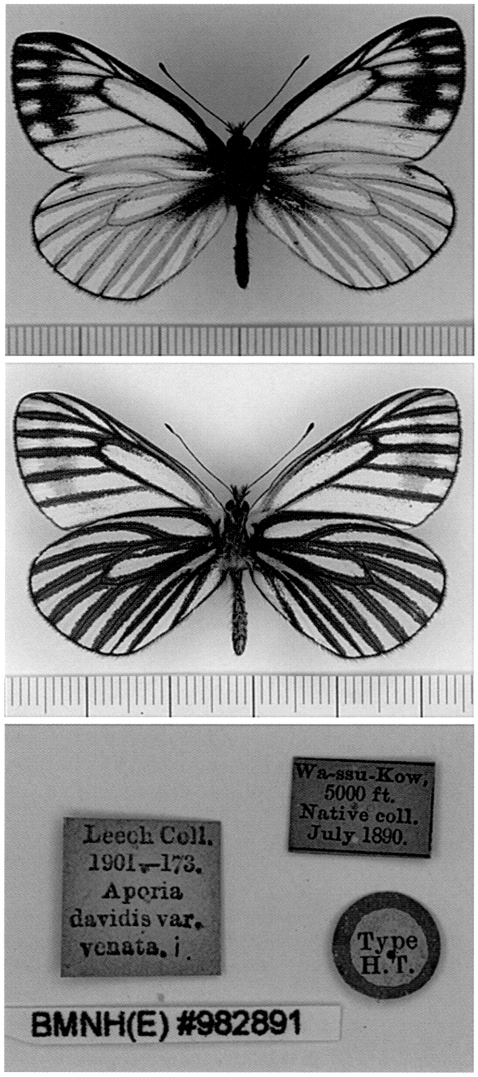

Fig. 8. Type specimen of *Sinopieris. venata* Leech 1891 ♂ (©NHM).

Remarks

Pieris shangrilla resembles *Sinopieris davidis* (Fig. 5) or its forms/subspecies such as *S. davidis thibetana* (Verity 1907) (Fig. 6), *S. davidis diluta* (Verity 1911) (Fig. 7) and **S. davidis* ssp. (Fig. 2), but the new species has distinct characteristics as follows (The specimens of *P. shangrilla*, **S. davidis* ssp. and *S. dubernardi* were collected at exactly the same place in May.)

1) *S. davidis* has a black marking on hindwing upperside spaces 6 and 7, while they are pure white without any black markings in *P. shangrilla* (Figs 1, 2, 5 A). This characteristic is essential to distinguish *P. shangrilla* from *S. davidis* and all other taxa belonging to the genus *Sinopieris*.

2) Black scales along the cubital vein of hindwing upperside continue from basal area to vein 2 in *S. davidis,* while they are limited to within basal area in *P. shangrilla* (Figs 1, 2, 5 B).

3) *S. davidis* has a small space 7 at forewing underside, while *P. shangrilla* has no space 7 (Figs 1, 2, 5 C).

4) Basal area of hindwing underside space 1 is vivid yellow in *S. davidis*, while it is same ground color of pale yellow in *P. shangrilla* (Figs 1, 2, 5 D).

5) *S. davidis* has one or two black streak(s) on the cell of hindwing underside and the (upper) streak is thick and continue to vein 5, while *P. shangrilla* has two black streaks which attenuate and often disappeare within discal area and do not continue to vein 5 (Figs 1, 2, 5 E).

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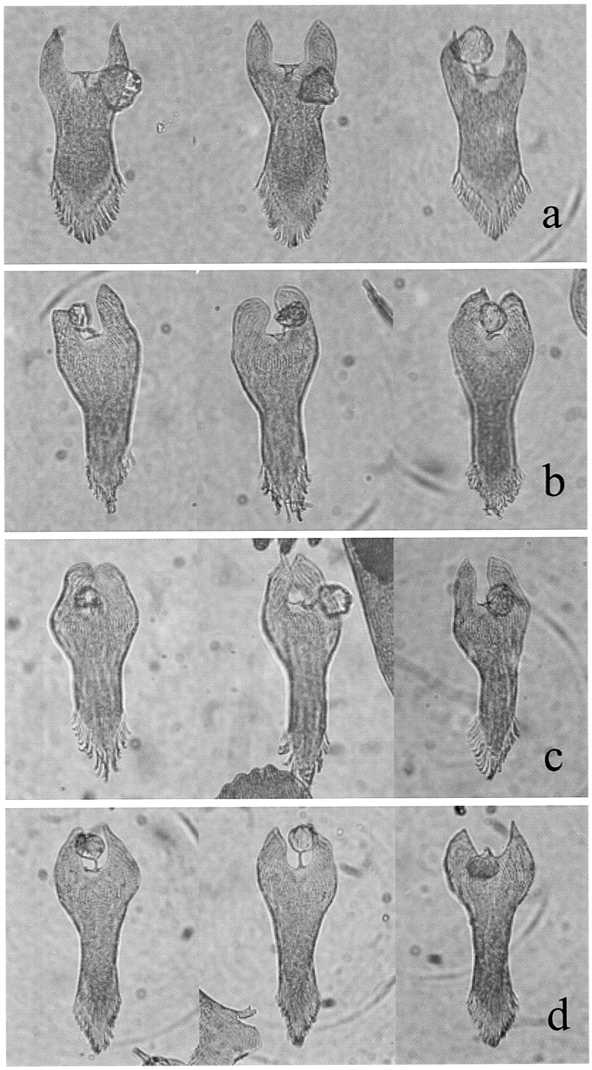

Fig. 9. Androconia of *shangrilla* (a), **davidis* ssp. (b), *venata* (c) and *dubernardi* (d).

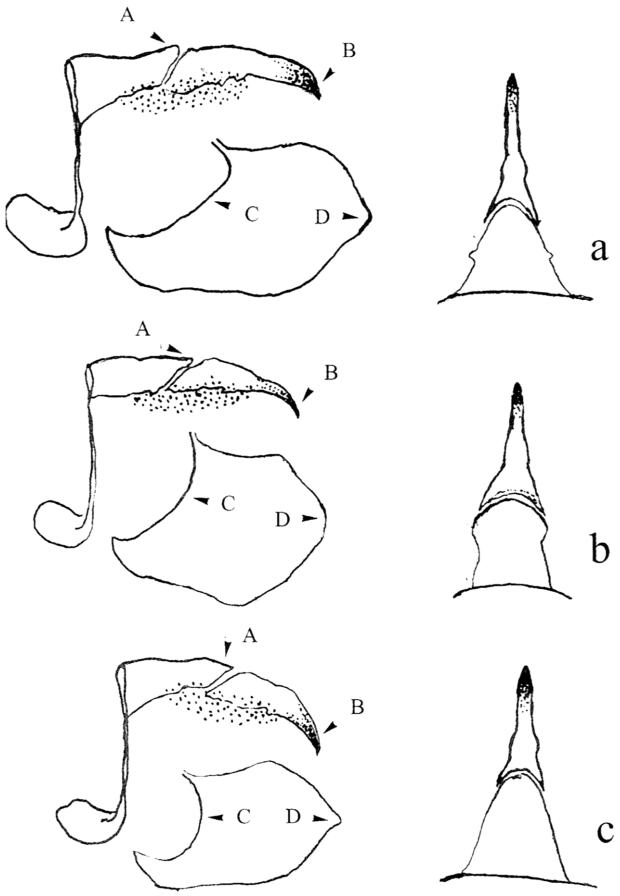

Fig. 10. Male genitalia of *Pieris shangrilla* (a), **Sinopieris davidis* ssp. (b) and *S. venata* (c).

Comparing to *S. venata* (Figs 3, 8), the following distinct characteristics are observed in addition to all of the abovementioned characteristics 1) The ground color of the hindwing underside is rich lemon yellow in *S. venata*, while it is pale yellow in *P. shangrilla* and *S. davidis*. 2) The venation is broadly bordered with black scales in *S. venata*.

Recently, we found illustrations of *P. shangrilla* in an old book and a photograph, where *P. shangrilla* were introduced as *S. davidis* or its related taxa as follows:

1. Verity (1905-1911) introduced some specimens of *davidis* and its related taxa, one of which was identified as *shangrilla* (Fig. 15 = PL. XXX-46: coll. Obth.) by distinct characteristics on the upperside hindwings. Verity included *shangrilla* as *davidis* var. *venata*. The specimen was collected at Lou-tse-Kiang (Lu-tse-Kiang = Nu Jiang) near Zhongdian district (Fig. 11).
2. A photograph, taken by Mr Nigel Peace of NHM in early June 2012, introduced *Pieris shangrilla* as *Sinopieris davidis* (Fig. 16). The photograph was taken at Weixi an altitude of 1,780 m, along the Lancang-Jiang (Mekon) and also very close to the Zhongdian district as well (Fig. 11).

Habitat and distribution

The type locality of *P. shangrilla* is a valley nearby Zhongdian in Yunnan Province of China (altitude of 3,100 m). Fig. 11 shows the type locality of *P. shangrilla* "Zhongdian District (Red point)" as well as the type localities of *S. davidis* and its related taxa (Blue points),

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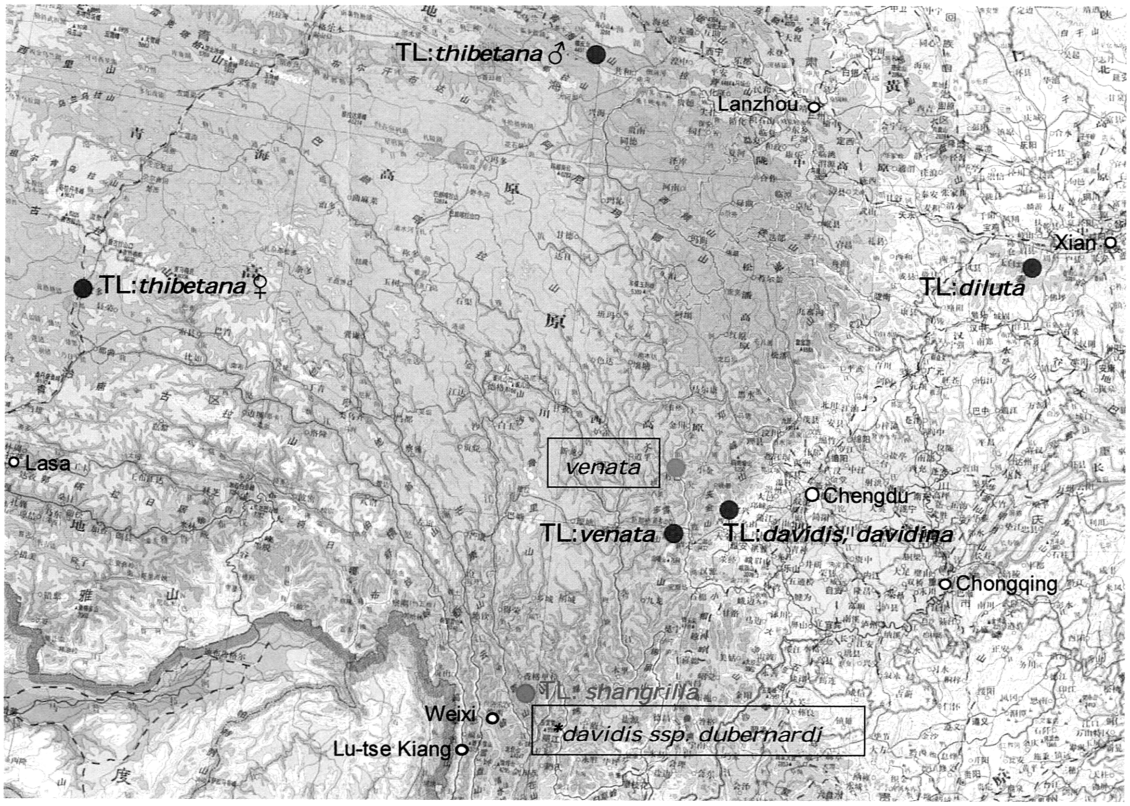

Fig. 11. Type localities of *Pieris shangrilla*, *Sinopieris davidis* and the related taxa.

and the collecting places of the specimens (enclosed).

We consider that *P. shangrilla* may occurs in a limited area around Zhongdian district of East Tibet from May to early June, while *S. davidis*, *S. venata* and/or its related taxa are broadly distributed in the Tibet Mts. including Tibet Autonomous Region, Qinghai, Gansu, Shaanxi, Sichuan, and Yunnan Provinces and occur from May to July. Or, *P. shangrilla* might have been treated as *S.davidis* as it appeared to have been confused with *S. davidis* for more than a century.

Discussion

At first sight, we considered that *Pieris shangrilla* might be within intraspecific variations of *Sinopieris davidi*s. But after careful observation of its wing markings, androconia and male genitalia in every detail, we came to recognize the distinct morphological characteristics of *shangrilla* stated in the description above. The photograph of the type specimen in NHM (Fig. 5) gave us a great help to identify *davidis* with confidence. In addition to ssp. *davidis* and **davidis* ssp., we examined two forms/ subspecies of *davidis* described by Verity as follows:

1) *davidis* (f.) *thibetana* Verity, 1907 (Type locality is Koukou-nor = Qinghai South Mts. for ♂ and Amdo for ♀ in Tibet), which is now treated as a form/synonym of *davidis* except Ziegler (2012), who treats *thibetana* as a subspecies of *davidis*. Verity (1907) described *thibetana* as a copy of ssp. *davidis* with smaller wing expanse. **Davidis* ssp. is probably *thibetana*, in terms of wing expanse, wing markings and season of occurrence (Fig. 6).
2) *davidis* (f.) *diluta* Verity, 1911 (Type locality is Taipaishan in Shaanxi Province of China), which is treated as a subspecies of *davidis* by Chou (2000) and Ziegler (2012). According to Verity (1911), *diluta* has less blackish wing design comparing to ssp. *davidis* (Fig. 7).

We also examined *venata* Leech 1891, by both photograph of type specimen (Fig. 8) and actual specimens collected near its type locality (Fig. 3). *Venata* (Type locality is Ta-chen-lu, the former name of Kangding in Sichuan Province) was originally described by Leech (1891) as the spring generation of *davidis* under the genus *Aporia*, but later treated as a distinct species by Röber (1906) and Ziegler (2012). Chou (2000) treated *venata* as a synonym of *davidis*.

The differences of *venata's* wing markings from *davidis*

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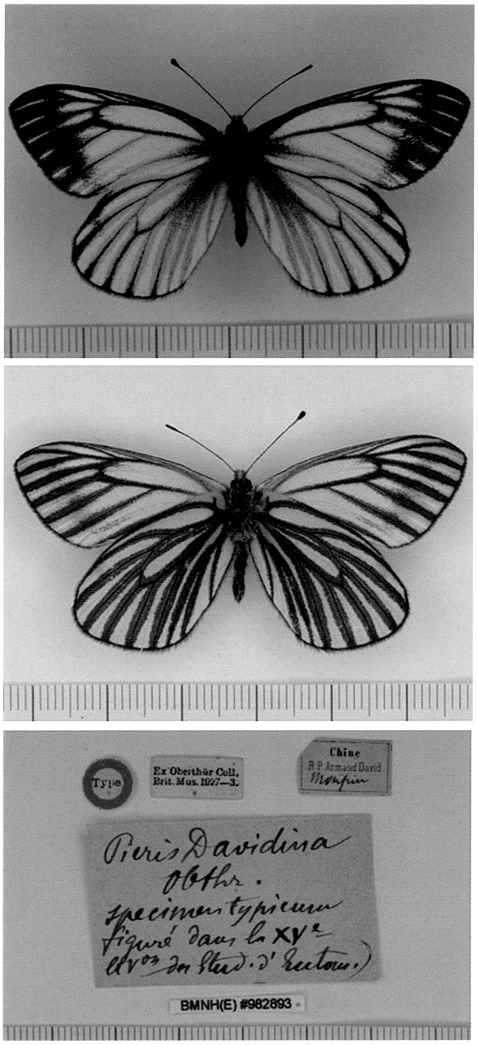

Fig. 12. Type specimen of *Sinopieris davidina* Oberthür, 1891 ♂ (©NHM)).

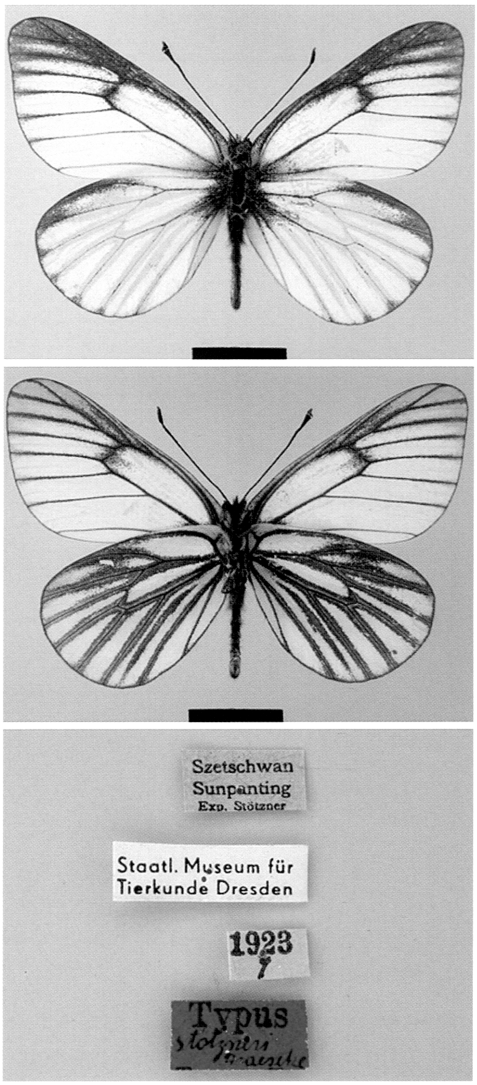

Fig. 13. Type specimen of *stotzneri* Draeseke 1924 ♂ (© SNS in Dresden).

and its subspecies consist only in a richer yellow ground color and thicker black venation on the hindwing underside. *Shangrilla* is more similar to *davidis* than to *venata*.

Furthermore, we examined *davidina* Oberthür 1891 (Fig. 12), which had been treated by Leech (1892-1894) and Verity (1905-1911) as a synonym of *venata* until Huang (2003) temporarily treated it as an independent species under the genus *Sinopieris*. Chou (2000) did not refer to *davidina* at all. The type locality was the same as *davidis* at Moupin. After careful examination, all distinct morphological characteristics of *shangrilla* vs. *davidis* and vs. *venata* were applied except the small space 7 of forewing underside.

The last comparison was vs. *stotzneri* Draeseke 1925 (Fig. 13), which was treated by Huang (2003) as an independent species under the genus *Sinopieris*.

Judging from the photograph of the type specimen, we felt that *stotzneri* might be included into the genus *Aporia* rather than *Sinopieris* or even *Pieris*. Bruna *et al*. (2004) introduced *stotzneri* under the genus of *Aporia* as "*Aporia martineti* ab. *stotzneri*".

Other than the above taxa, we could not find any taxa similar to *shangrilla*.

We looked into the description of the genus *Sinopieris* Huang 1995 in every detail as follows:

(a) Wing shape rather narrow as in *Aporia*, (b) discoidal cell slightly longer than 1/2 costa, (c) all veins slightly marked with black, (d) black discoidal bar of forewing conspicuous, (e) Both sexes bear an apical marking on

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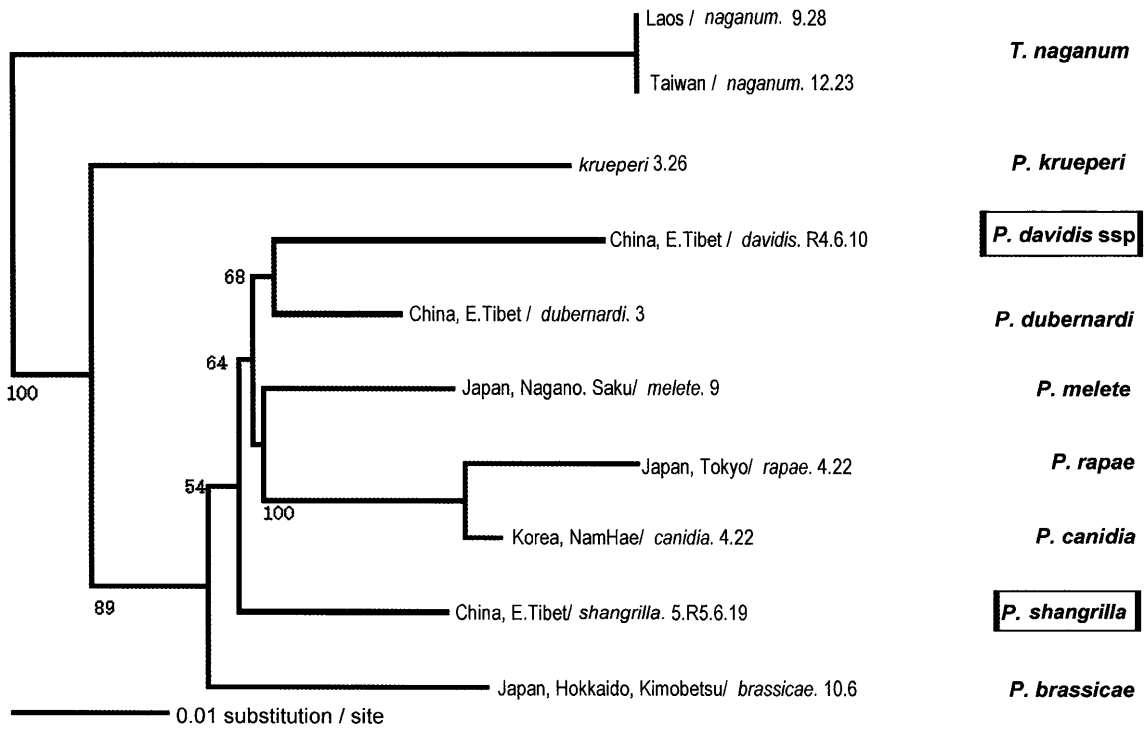

Fig. 14. NJ Tree by *mt*DNA -16SrRNA gene.

the hindwing space 6, (f) female upperside has postdiscal blackish markings in at least forewing space 1 to 3, and sometimes also in the hindwing postdiscal area.

On this basis we concluded that, in accordance with the description item (e), *shangrilla* could not be included in the genus *Sinopieris*.

When we observed the androconia, we were convinced that *shangrilla* was a distinct species and could be easily distinguished from **davidis* ssp., *venata* and *dubernardi*. Androconia are laid on the male's forewing cell and emit a distinct sexual odor. Each consists of a wrench-shaped lamina and blackish scent-bag connected to the center of the lamina by a slender pipe of vein. The shape of lamina and the size of scent bag can help to identify species. The lamina of *shangrilla* was broad and 'wrench-shape' very broad, similar to *dubernardi* or *extensa*, while the lamina of *davidis* and/or *venata* had narrow 'wrench-shape' (Fig. 9b-c) resempling *gyantsensis* and the *napi*-Complex. The size of the scent bag was large enough to identify *shangrilla* from **davidis* ssp., *venata*, *dubernardi* (Fig. 9).

The male genitalia can also distinguish *shangrilla* from both **davidis* ssp. and venata by the size and shape of uncus and valva (Fig. 10).

Notes

**Sinopieris davidis* ssp.: The butterfly obtained from the same place as *Pieris shangrilla* and *Sinopieris dubernardi*, which we considered as *Sinopieris davidis thibetana*. In this paper, *shangrilla* shall be read as *Pieris shangrilla* sp. nov.

Conclusion

In addition to the distinct morphological characteristics stated above, the phylogenetic relationship based on mtDNA genes (16SrRNA) clearly indicated that the status of *shangrilla* has its own molecular linage and no close relationship with *davidis* and/or even *dubernardi* (Fig. 14). This coincided with the results of the morphological study that *shangrilla* did not belong to the genus *Sinopieris*. Based on the distinct morphological characteristics and phylogenetic analyses (Fig. 14), we recognize *shangrilla* as a new independent species under the genus *Pieris*. Within the genus Pieris, similarity of wing markings do not always mean affinities in molecular phylogeny. Like the relationship between *rapae* and *pseudonapi* in Europe, *extensa* and *erutae* in West China or *melete* and *japonica/nesis* in Japan, *shangrilla* is found in the Tibet Mts. with *davidis* or its related species/subspecies as a distinct species,

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although their wing markings are very much alike. *Pieris shangrilla* sp. nov. will have common names as "シャングリラシロチョウ" in Japan, and as "香格里拉粉蝶" in China.

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摘　要

中国雲南省北部に生息する*Pieris*属の1新種の記載（鱗翅目，シロチョウ科）（田所輝夫・新川　勉・王　敏）

東アジアの*Pieris*属ならびにその近縁種について著者らが行った最近の現地調査並びに分子系統分析の結果，*Sinopieris davidis*及びその近縁（亜）種に酷似した新しい蝶を発見するに至った．その蝶は東チベットにおいて2009年及び2012年の5月に採集されたものである．翅紋・発香鱗・生殖器を精査した結果，その蝶は独特の形態的特徴をもつことが明らかになった．

Huang (1995)は新種*Sinopieris gongaensis*をタイプ種として新属*Sinopieris*を記載し，*dubernardi*グループ（*dubernardi*及び近縁種/亜種）を*Pieris*属/*Pontia*属から*Sinopieris*属に属変更した．ただし，*gongaensis*は後に*(davidis) venata*のシノニムとされた．それ以後*dubernardi*, *davidis*, *venata*及びそれらの関連タクサが*Sinopieris*属に含まれることになった．

ところがこの新しい蝶は*Sinopieris davidis*に酷似しているものの，翅紋の一部が*Sinopieris*属の記載に適合しない．また発香鱗や♂生殖器からもこの蝶が*davidis*や*dubernardi*とは全く異なることが示唆された．さらに*mt*DNA因子（16 SrRNA）の分子分析の結果からも，独自の分子系統を持ち*davidis*や*dubernardi*とは全く違う系統であることが示唆された．

以上のように形態的特徴及び分子系統分析の結果にもとづいて，この蝶を*Pieris*属の新種*Pieris shangrilla* sp. nov.（和名：シャングリラシロチョウ）として記載した．Pieris属においては翅紋の類似性が必ずしも系統上の類縁性を意味しない．たとえば欧州における*rapae*と*pseudorapae*との関係，中国西部における*extensa*と*erutae*との関係あるいは日本における*melete*（スジグロシロチョウ）と*japonica*（ヤマトスジグロシロチョウ）との関係同様，翅紋が非常に類似していながらも*shangrilla*は*davidis*あるいはその近縁種とは系統が異なる別種としてチベット山脈で共存していると考えられる．

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